## REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and the following comments.

### I. Status of the Claims

Claims 1-26 are cancelled previously. Claims 39 and 40 have been amended to set forth the subject matter more clearly. Because the amendments do not introduce any new matter, Applicants respectfully request entry of this amendment. Upon entry, claims 27-60 will be pending, with claims 42-60 withdrawn from examination.

### II. Species Election

Applicants elected SEQ ID NO: 2, which corresponds to chicken troponin C, in the prior response. However, Applicants inadvertently misstated that the troponin type was human troponin C. Although Applicants respectfully disagree with the Examiner that SEQ ID NO: 2 will not be specifically examined, Applicants understand that the species election requirement was made to facilitate the examination and that upon allowance of a generic claim, a reasonable number of species will be rejoined for examination in the present application.

### III. Rejection of Claims under 35 U.S.C. §112, second paragraph

Claims 39 and 40 are rejected under 35 U.S.C. §112, second paragraph, for allegedly being indefinite because the claims recite overlapping ranges. The claims at issue have been amended to recite alternative ranges by the conjunction "or." Accordingly, Applicants respectfully request withdrawal of the rejection.

### IV. Rejection of Claims under 35 U.S.C. §103(a)

Claims 27-41 are rejected under 35 U.S.C. §103(a) for allegedly being obvious over U.S. Patent Application Publication No. 2003/0017538 by Miyawaki et al. ("Miyawaki") in view of

Gahlmann et al., *J. Biol. Chem.*, 265(21): 12520-12528 (1990) ("Gahlmann"). Applicants respectfully traverse the rejection.

**A. <u>The $Ca^{2+}$-binding polypeptide of the claimed invention is distinguishable from that of the cited art.</u>**

The claimed invention as prescribed by claim 27 is directed to a polypeptide comprising a FRET donor molecule, a polypeptide having a certain level of identity to human troponin C, chicken skeletal muscle troponin C, or drosophila troponin C isoform 1; and a FRET acceptor molecule.

Thus, the invention requires a certain form of troponin C as a calcium binding moiety to yield a fully functional calcium-binding ratiometric FRET-indicator protein. The three best performing indicator constructs used are variants of human TnC, chicken skeletal TnC and Drosophila TnC isoform 1 as calcium binding moiety. *See* published application, at page 3, paragraph [0017].

In contrast, the prior-art FRET indicators such as those described by Miyawaki are of the "Cameleon"-type. That is, a calcium-binding polypeptide (calmodulin in the case of Cameleon) always has to be used in combination with a specific binding partner (the calmodulin target peptide M13 in the case of Cameleon) to yield a fully functional indicator. *See* the published application, paragraph [0002]; and Miyawaki, paragraph [0102], the last five lines, and at page 10, right column, lines 2-5. The reason is that the "Cameleon"-indicators essentially detect and quantify an interaction between the calcium-binding polypeptide (calmodulin) and its target peptide (M13).

This teaching of an obligatory calcium-binding protein/target protein pair is also expressed by Miyawaki, at page 6, paragraph [0102], which describes the prior art FRET indicator Cameleon, i.e. calmodulin in combination with a target polypeptide. The following paragraph [0103] lists further potential calcium-binding proteins, and paragraph [0104] explains

how suitable target peptides for those calcium-binding proteins can be found in order to create functional FRET indicators.

Thus, although Miyawaki mentions troponin C among many other possibilities for calcium-binding polypeptides, there is a clear teaching that such a calcium binding polypeptide has to be used in combination with a suitable target peptide. This reflects the common knowledge in the field at the filing date of the present application that a calcium-binding polypeptide in a FRET indicator always had to be paired with an appropriate binding partner.

In contrast, the present inventors were the first to show that, unexpectedly, it is possible to create a calcium indicator that contains solely a certain type of troponin C as calcium-binding moiety and still responds very well to calcium stimuli, even in challenging cellular environments (*see* published application, at page 1, paragraph [0005], and at page 4, the beginning of paragraph [0028]). The indicators encompassed by the claimed invention, the three claimed troponin C types, when used alone, are proved to be superior to fusion constructs that require an additional target peptide, such as troponin I. *See* Figure 2 of the application, which demonstrates the constructs comprising troponin I and their respective performance.

Accordingly, the claimed invention provides a series of novel calcium indicators containing only a specific troponin C type as calcium-binding moiety without the need for any additional binding peptides. Unexpectedly, these constructs performed even better than a prior-art combination of a calcium-binding protein and a binding peptide.

It is unexpected, in view of the teachings of the cited art, that the specific combinations of the claimed invention, i.e., a FRET donor and acceptor pair together with variants of either human troponin C, chicken skeletal muscle troponin C, or drosophila troponin C isoform 1 as calcium-binding polypeptide, produced superior results when compared to the prior art. More specifically, no functional labelings of either membranes, pre- and post-synaptic structures or calcium channels had been reported for the prior-art Calmodulin based calcium indicators. In

contrast, the present inventors demonstrate that the novel indicator "TN-L15" of the claimed invention exhibited superior functionality under the same conditions, whereas the prior-art "Yellow Cameleon" represented by Miyawaki was not functional when targeted to the cell membrane. *See* published application, at page 13, Example 4 and Figures 10 and 11.

**B. The cited art fails to provide any reason to use generic troponin C as the $Ca^{2+}$-binding polypeptide**

The examiner acknowledges that Miyawaki fails to explicitly teach human, chicken or Drosophila troponin C (*see* Office Action, at page 7, last paragraph) but alleges that Miyawaki "provides explicit teaching, suggestion and motivation" to use generic troponin C as a replacement for the prior-art calmodulin as a calcium-binding polypeptide (*id.*, page 9, last full paragraph). Applicants respectfully disagree.

**First**, as discussed above, Miyawaki suggests that a calcium-binding polypeptide must be used in combination with a suitable target peptide. Miyawaki does not teach or suggest the use of a calcium-binding polypeptide ***alone***.

**Second**, Miyawaki merely provides a laundry list of calcium-binding proteins that might be used as calcium-binding polypeptides in a FRET pair. *See* page 6, paragraph [0103]. There is no teaching or suggestion that a troponin C should be preferably selected. Furthermore, a skilled person would not have been motivated to choose troponin C because troponin C is not a cytosolic protein, but a protein that, in its natural form, occurs only in a tight protein complex as a part of the troponin complex in muscle. *See* Gahlmann, page 12520, left column, last two lines, through right column, first two lines.

It was therefore doubtful whether a single molecule of muscle troponin C would fold correctly and maintain its crucial capabilities for calcium-binding and conformational change in cytosolic environments. Thus informed by Gahlmann, a skilled person would choose a protein

that was known to be of cytosolic origin and therefore more likely to be unaffected by the cytosolic environment when expressed within a cell.

**Third**, the biological function of troponin C would have discouraged a skilled person from selecting troponin C as the $Ca^{2+}$-binding polypeptide in view of many polypeptides disclosed by Miyawaki. The role of troponin C in the muscle troponin complex is that of a protein switch which undergoes a rapid conformational change on the binding of calcium, thereby initiating the contraction of muscle fibres (*see* Gahmann, page 12520, the paragraph bridging the columns). A skilled person could not have expected this calcium binding and fast-switching conformational change of a specific form of troponin C (such as human TnC, chicken skeletal TnC or Drosophila TnC) to occur in a graded, stepwise fashion, which is a prerequisite if one contemplates using such a protein in a calcium indicator. Nevertheless, the present inventors made a novel and surprising discovery that if a specific form of troponin C, namely human troponin C, chicken skeletal muscle troponin C, or drosophila troponin C isoform 1, was used as a calcium-binding moieties in a FRET-polypeptide, the fluorescence signal emitted by these indicators resulted in graded responses reflecting a steady increase in calcium concentration and therefore could be readily calibrated to a wide range of different calcium concentrations. Such calibration curves are indicated in Example 2 and Figure 4A in the specification.

### C. There was no reasonable expectation of success when the teachings of Miyawaki and Gahlmann are combined.

The examiner asserts that since Gahlmann teaches a calcium-binding capacity of the specific form human troponin C, the person of ordinary skill in the art would have been motivated to replace the generic troponin C of Miyawaki with the specific human troponin C of Gahlmann, i.e. the rationale of substituting one known, equivalent element for another to obtain predictable results. *See* Office Action, the paragraph bridging pages 9 and 10. In particular, the Examiner's position appears to be that the selection of human troponin C would have been obvious in view of Gahlmann because human troponin C would have utility in a system using

human cells and would be functionally equivalent to the generic troponin C as taught by Miyawaki.

However, there would not have been a reasonable expectation of success, as the Examiner asserts, when the teachings of Miyawaki and Gahlmann are combined. More specifically, one skilled in the art would not have been able to select a generic troponin C from the list disclosed by Miyawaki and then replace the generic troponin C with human troponin C which is taught by Gahlmann. As discussed in the foregoing paragraphs, the fact that troponin C is not a cytosolic protein, but a part of the muscle troponin complex, made it very questionable whether any specific form of troponin C (human, chicken, or drosophila) would still show the necessary correct folding, calcium-binding, and conformational change in a cytosolic environment. Moreover, it was unpredictable that any specific form of troponin C would show a slow, graded conformational change upon calcium binding, given their natural role as a fast protein switch.

In view of the foregoing, Applicants respectfully request withdrawal of the rejection.

## CONCLUSION

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment instructions in EFS-Web being incorrect or absent, resulting in a rejected or incorrect credit card transaction, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of

papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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